Quantitative selection and parallel characterization of aptamers.

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Public Summary:
Aptamers are promising affinity reagents that are made up of a string of nucleic acids that bind tightly to their targets. They are useful for high-throughput discovery, as they are chemically synthesized and discovered via completely in vitro selection processes. Recent advancements in selection, sequencing, and the use of modified bases have improved aptamer quality, but the overall process of aptamer generation remains laborious and low-throughput. This is because binding characterization remains a critical bottleneck, wherein the affinity and specificity of each candidate aptamer are measured individually in a serial manner. To accelerate aptamer discovery, we devised the Quantitative Parallel Aptamer Selection System (QPASS), which integrates microfluidic selection and next-generation sequencing with in situ-synthesized aptamer arrays, enabling simultaneous measurement of affinity and specificity for thousands of candidate aptamers in parallel. We used QPASS to select aptamers that bind tightly to the human cancer biomarker angiopoietin-2 (Ang2), which might be useful in screening patients for early detection of cancers. This novel approach should have broad application in the development of new drugs as well as new diagnostics for cancer and other diseases.

Scientific Abstract:
Aptamers are promising affinity reagents that are potentially well suited for high-throughput discovery, as they are chemically synthesized and discovered via completely in vitro selection processes. Recent advancements in selection, sequencing, and the use of modified bases have improved aptamer quality, but the overall process of aptamer generation remains laborious and low-throughput. This is because binding characterization remains a critical bottleneck, wherein the affinity and specificity of each candidate aptamer are measured individually in a serial manner. To accelerate aptamer discovery, we devised the Quantitative Parallel Aptamer Selection System (QPASS), which integrates microfluidic selection and next-generation sequencing with in situ-synthesized aptamer arrays, enabling simultaneous measurement of affinity and specificity for thousands of candidate aptamers in parallel. After using QPASS to select aptamers for the human cancer biomarker angiopoietin-2 (Ang2), we in situ synthesized arrays of the selected sequences and obtained equilibrium dissociation constants (Kd) for every aptamer in parallel. We thereby identified over a dozen high-affinity Ang2 aptamers, with Kd as low as 20.5 +/- 7.3 nM. The same arrays enabled us to quantify binding specificity for these aptamers in parallel by comparing relative binding of differentially labeled target and nontarget proteins, and by measuring their binding affinity directly in complex samples such as undiluted serum. Finally, we show that QPASS offers a compelling avenue for exploring structure-function relationships for large numbers of aptamers in parallel by coupling array-based affinity measurements with next-generation sequencing data to identify nucleotides and motifs within the aptamer that critically affect Ang2 binding.

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